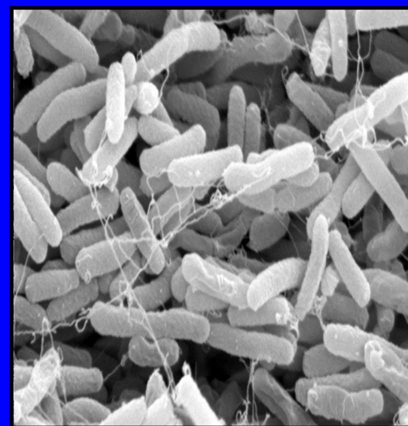
Microbial Defense Systems

Evolutionary & Ecological Perspectives

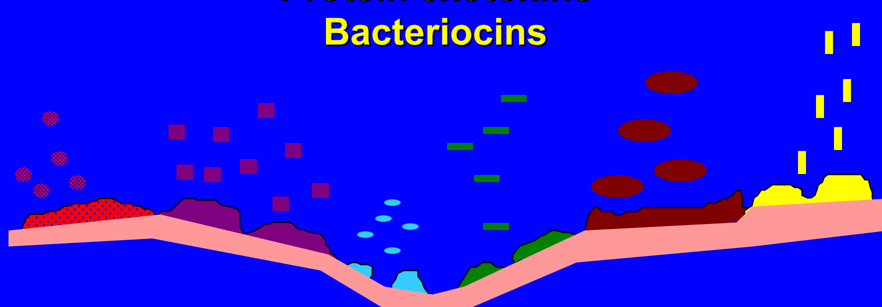


<u>*DOE Genomics Workshop*</u> *-*

What can genomics and population genetics tell us about bacterial species stability?

Microbial defense systems

Classical antibiotics
Metabolic by-products
Lytic agents
Protein exotoxins
Bacteriocins



(cross section of a mammalian large intestine)

Bacteriocins

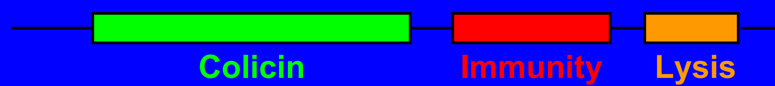
The most abundant and diverse microbial defense system

Escherichia coli	colicin
Pseudomonas aeruginosa	pyocin
Klebsiella pneumoniae	klebocin
Bacillus subtilis	subtilin
Staphylococcus epidermis	epidermin
Erwinia herbicola	herbicolacin
Lactobaccillus brevis	brevicin
Halobacteria	halocin

Colicins

- Toxins produced by and active against *E. coli* and other closely related bacteria
- Killing mechanisms
  - pore formation: A, B, E1, Ia, Ib, K, N, 5, 10
  - DNase: E2, E7, E8, E9
  - RNase: E3, E4, E6, DF13
  - inhibit protein synthesis: D, E5
  - inhibit murein biosynthesis: M

Colicin gene clusters



Colicin : killing function

Immunity : specific immunity to that colicin

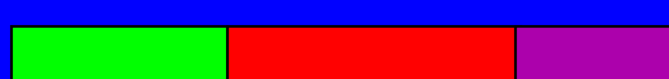
Lysis : lyse cell to release colicin

Colicin protein domain structure

Translocation

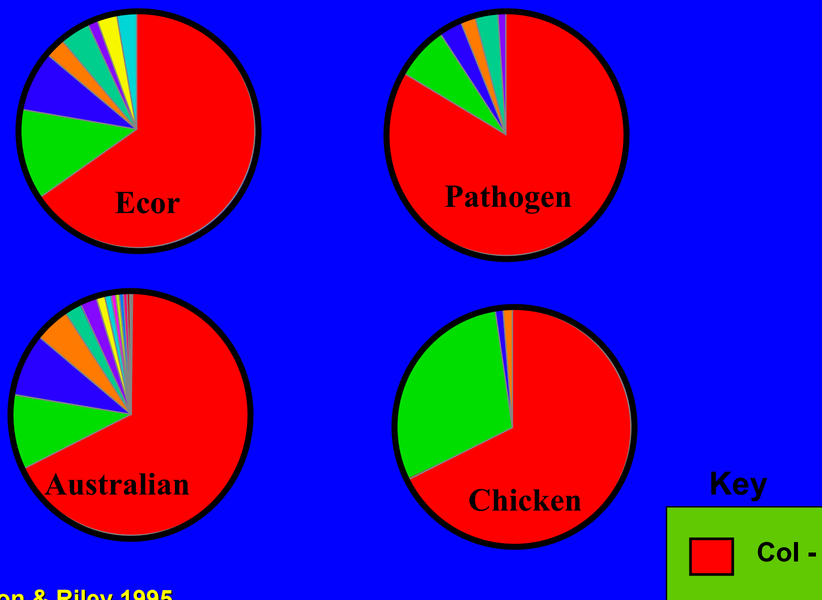
Receptor recognition

Killing and immunity binding



Colicin protein

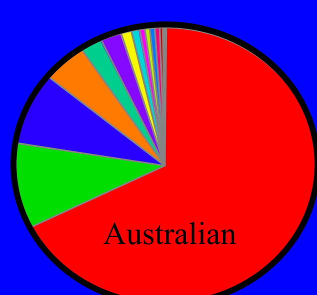
Colicin frequencies in nature



Gordon & Riley 1995

Colicin Evolution

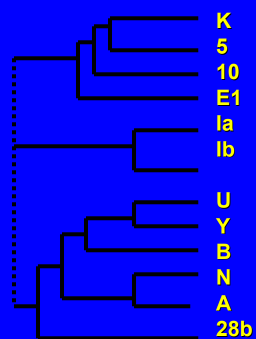
Why are there such high levels of colicin protein diversity?



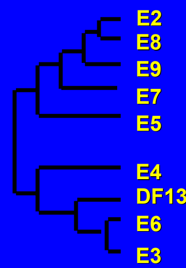
Riley, 1998

Colicin phylogenentic relationships

Pore former colicins

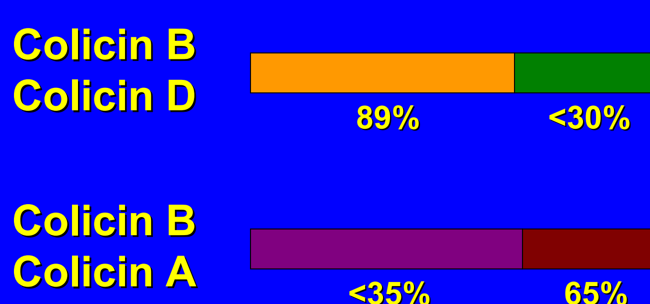


Nuclease colicins



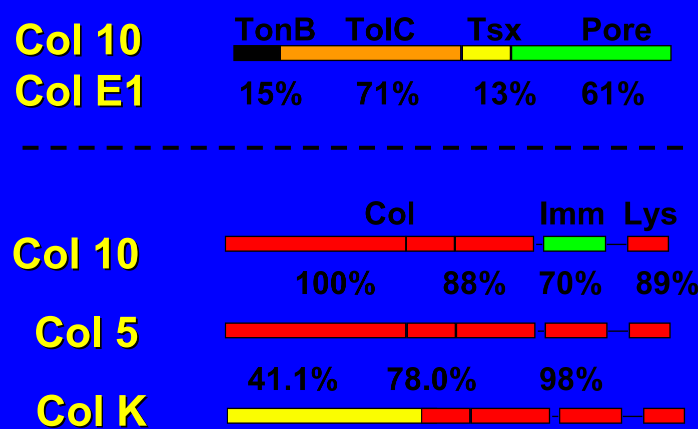
Riley, 1993

Pore former colicins diversify by domain swapping

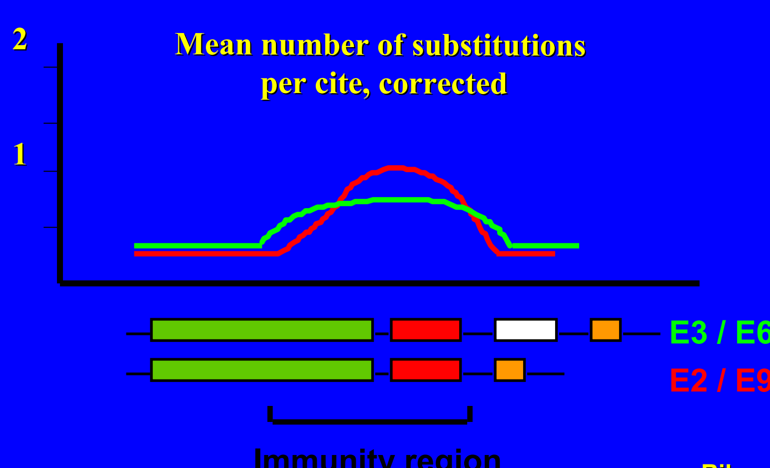


Braun et al. 1992

Recombination generates chimeric pore former proteins

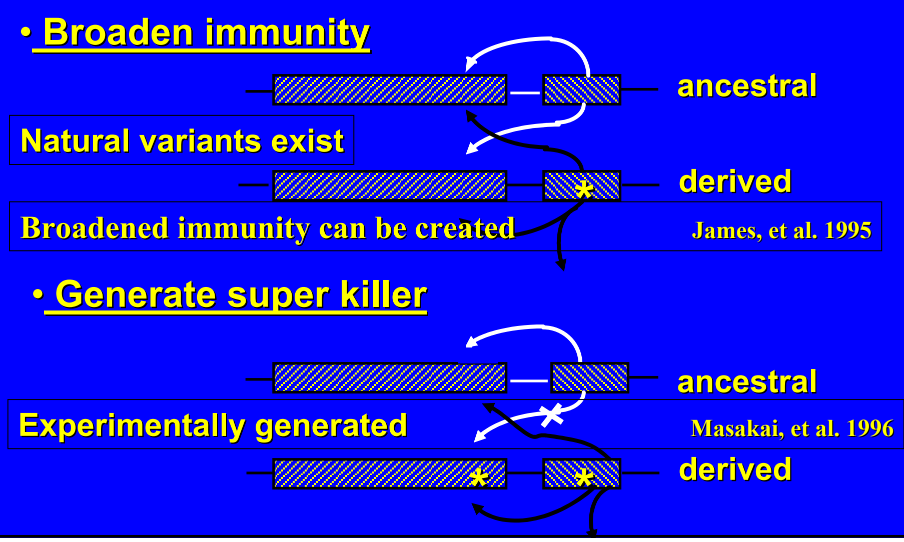


Nuclease colicins have a different pattern of substitution



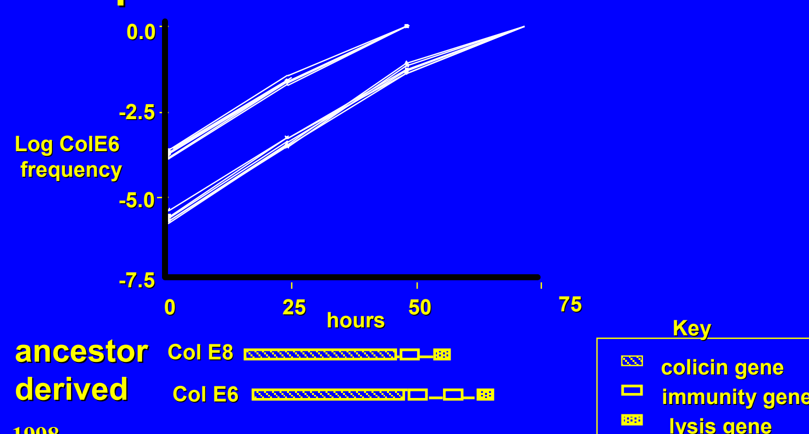
Riley, 1993

Nuclease colicins diversify through mutation & selection



Support for the diversifying selection hypothesis

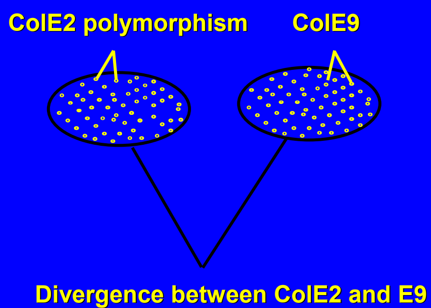
Experimental evolution studies

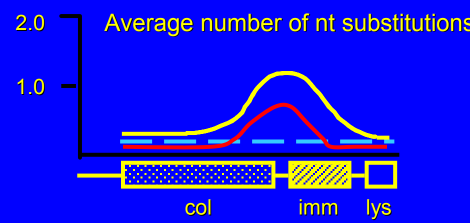


Tan & Riley, 1998

Support for the diversifying selection hypothesis

Nucleotide polymorphism and divergence



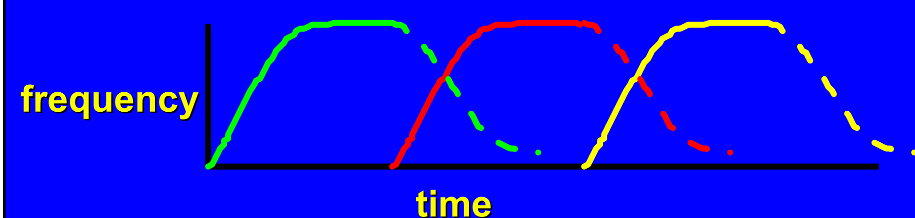


HKA test:

X^2 = 9.544, P < 0.005***

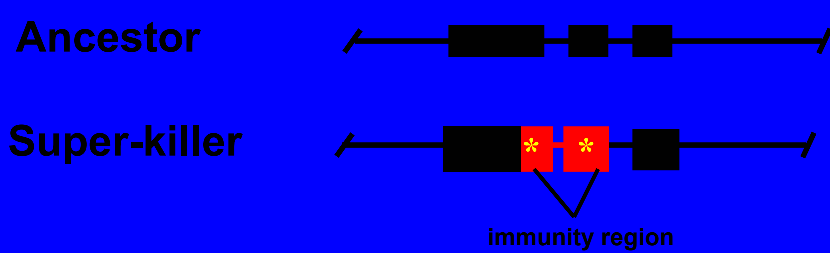
Tan & Riley, 1997

Diversifying selection in action



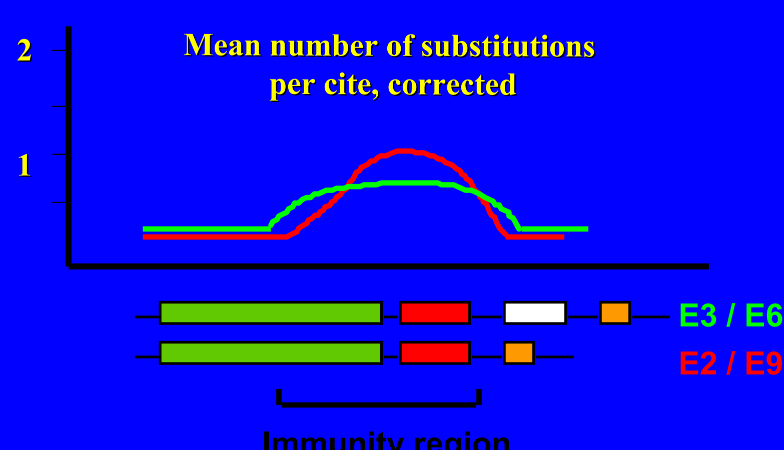
- Repeated rounds of fixation
- Migration reintroduces ancestral forms
- Recombination homogenizes parts of the colicin plasmids...

Recombination homogenizes nuclease colicin plasmids



- Recombination in immunity region is lethal (creates a mutational trap)
- Colicin plasmids diverge rapidly in the immunity region

Diversifying selection results in clustered divergence in immunity region



Two phases in the evolution of colicin diversity

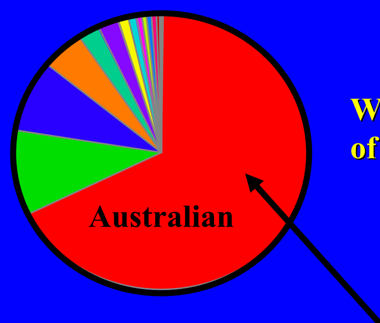
- Novel colicins created by diversifying selection
  - as seen for nuclease colicins
  - If successful, they become abundant in the population
- Once abundant, further diversity is created by diversifying recombination
  - as seen for pore former colicins

Mechanisms that generate colicin diversity are frequency dependent not colicin class dependent

- Colicin Y - pore former
  generated by point mutations
- Colicin E2 - nuclease
  generated by recombination

Colicin Ecology

What role do colicins play in natural populations?

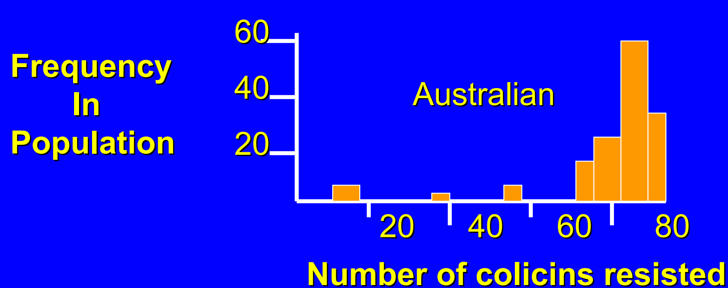


Who are the targets of colicin killing?

Are the red cells sensitive to colicins?

Riley & Gordon 2000

High levels of colicin resistance



Most red cells are resistant
to the co-segregating colicins

Evolution of Colicin Resistance

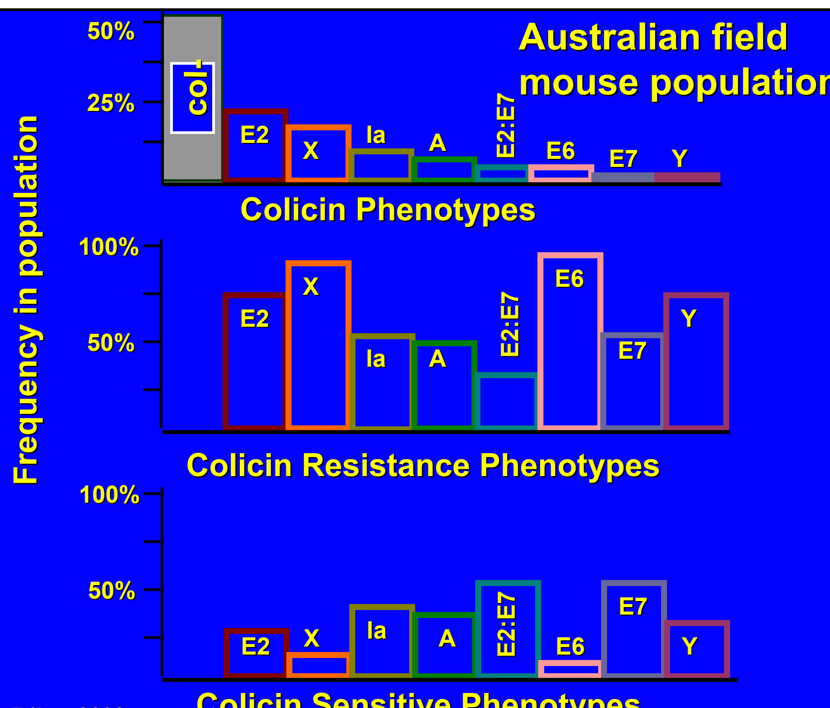
- 10^{-7} Resistance mutation rate
- All resistance mutations are pleiotropic

Multi-resistance can be maintained
with a single colicin

- There is a cost to resistance

Altering a cell surface receptor or
translocation system decreases a
cells growth rate

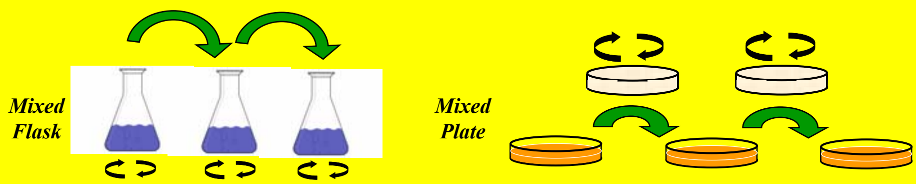
Feldgarden & Riley, 2000



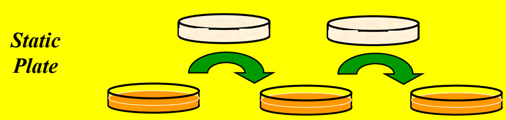
Gordon & Riley, 2000

Experimental ecology

Mixed Treatments

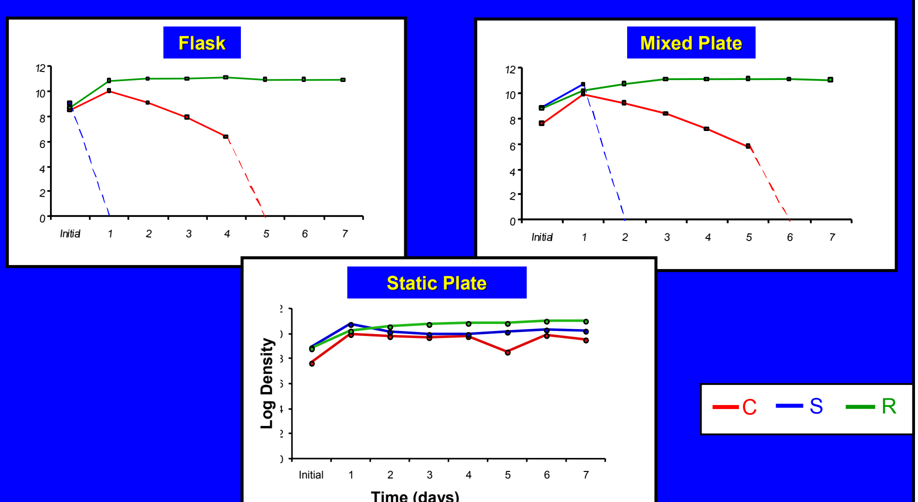


Spatially-Structured Treatment



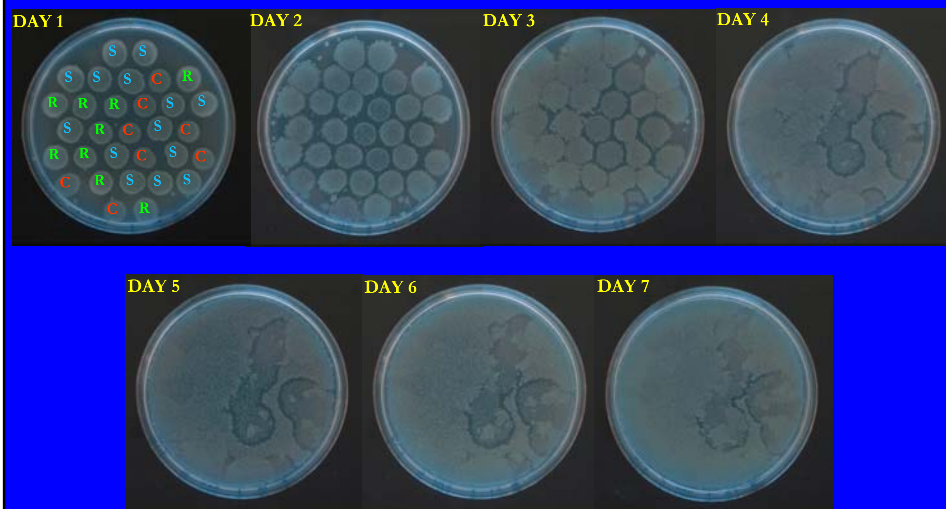
Kerr, et al. In press

Experimental results



Kerr, et al. In press

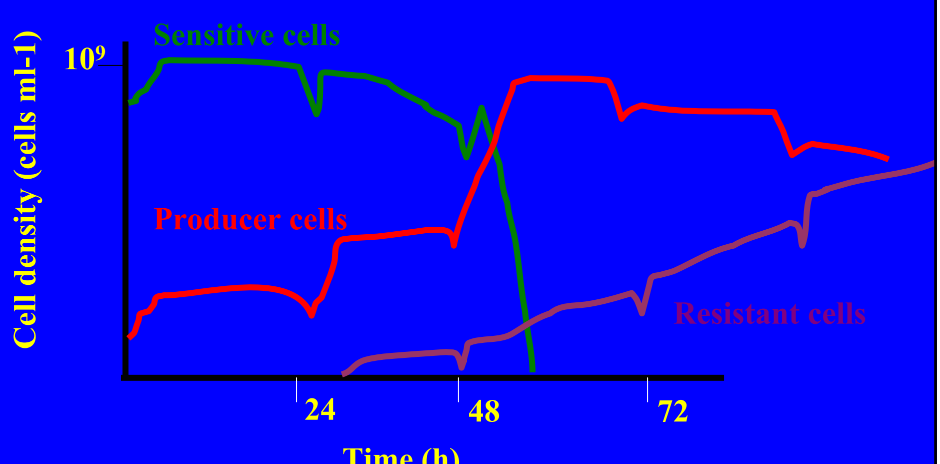
"Chasing" was observed!



Kerr, et al. In press

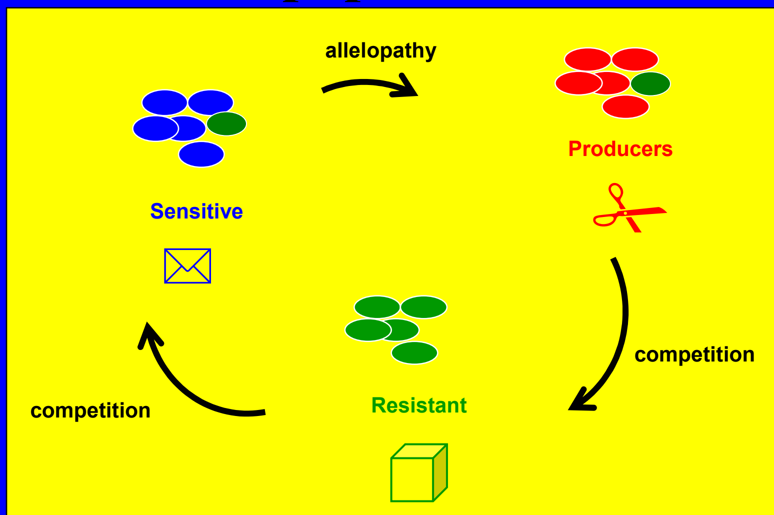
Theoretical ecology

Computer simulation of mixed flask



Gordon & Riley, 1999

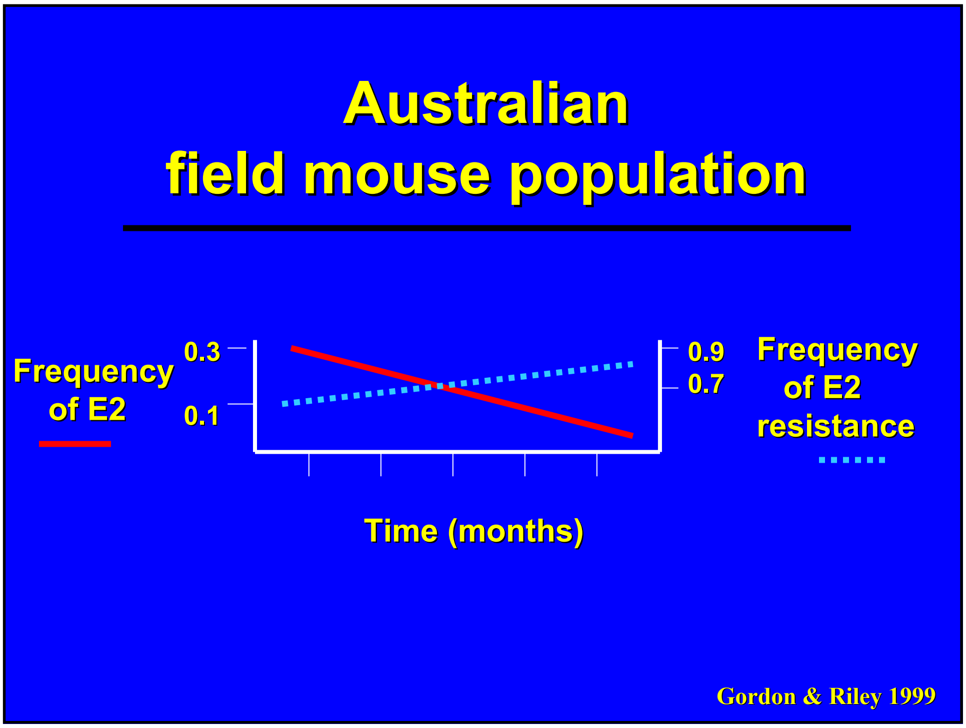
A microbial game of rock-paper-scissors

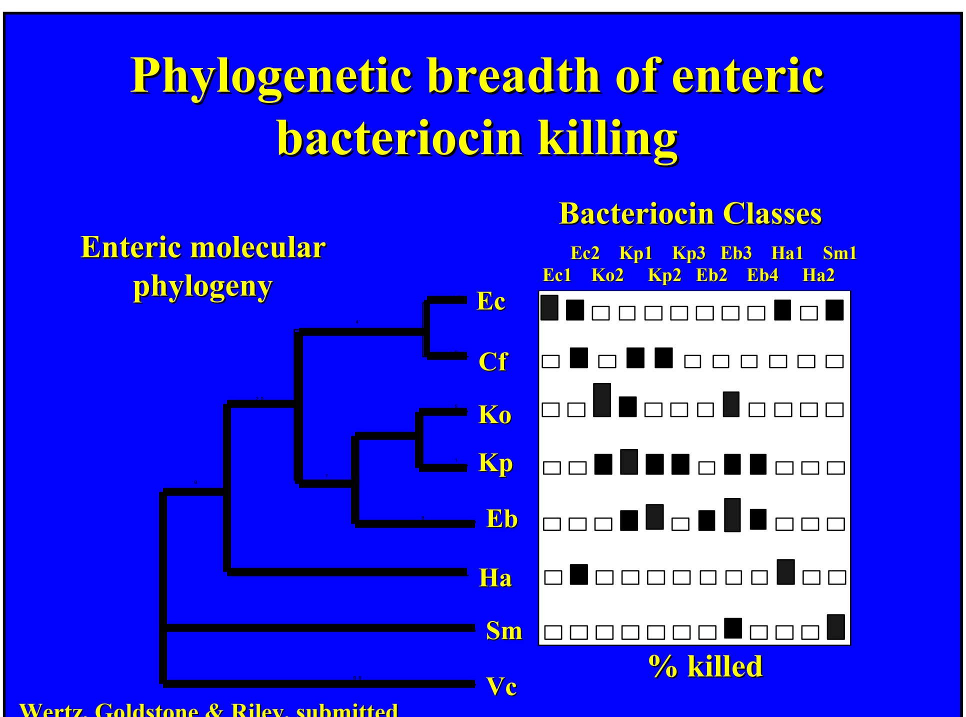


Kerr, et al. In press

Model captures features seen in natural populations

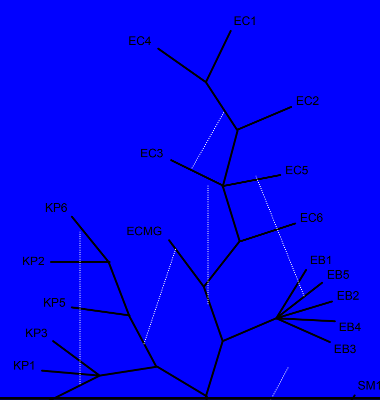
- Combination of sensitive, producer and resistant strains will be present
- Producer & resistant strains will dominate
  - Sensitive strains are rapidly displaced
- Continual flux in sensitive, producer and resistant strains expected
  - Mouse survey shows flux

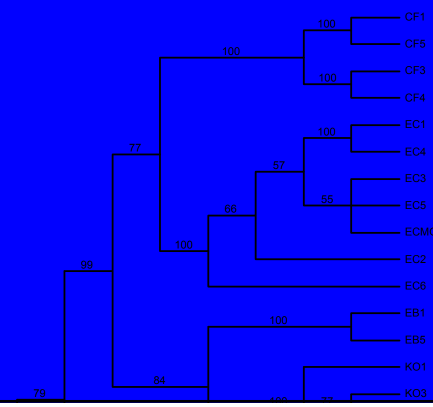

Australian
field mouse population
Frequency of E2
0.3
0.1
0.9
0.7
Frequency of E2 resistance
Time (months)
Gordon & Riley 1999


Phylogenetic breadth of enteric bacteriocin killing
Enteric molecular phylogeny
Bacteriocin Classes
Ec2 Kp1 Kp3 Eb3 Ha1 Sm1
Ec1 Ko2 Kp2 Eb2 Eb4 Ha2
Ec
Cf
Ko
Kp
Eb
Ha
Sm
Vc
% killed
Wertz, Goldstone & Riley, submitted

Networks versus Trees?

- Should bacterial phylogenies be viewed as networks or dichotomously branching trees?
  - comparative genomics reveals abundant lateral transfer





Is there stability in bacterial evolution?

Are there long-lived bacterial lineages?

Are there bacterial species?

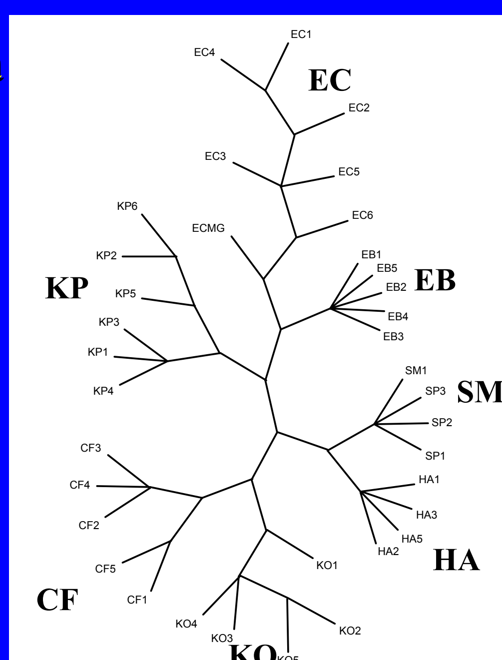
Bacterial species and the core genome hypothesis

- Lan and Reeves 2000, 2001
  - Core versus auxiliary genes
    - Core genes (aka housekeeping genes)
    - Auxiliary genes (PAI's, resistance, virulence factors, etc)
  - Biological Species Concept
    - Applied to bacteria: does recombination within a bacterial species happen more frequently than does lateral transfer between species for the core genome?
    - There have been no data sets designed to address this issue
    - UNTIL NOW!

*gap*A network for enteric bacteria

- Isolates from within a species always cluster genetically
- Clusters correspond to phenotype clusters (aka species clusters)

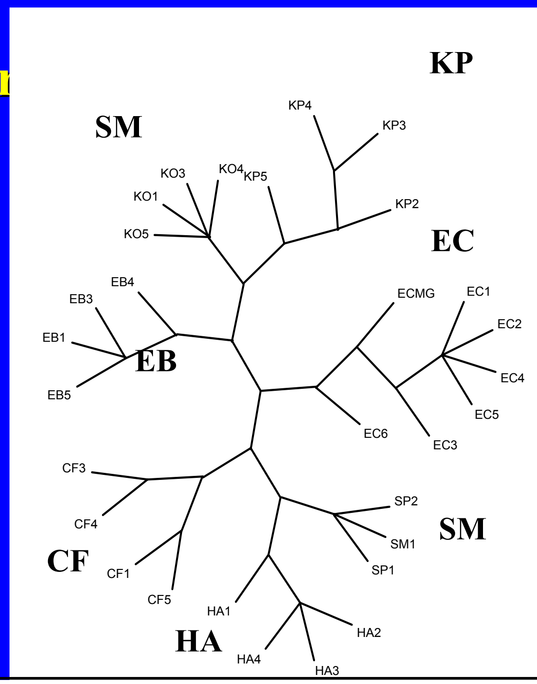
Wertz et al, submitted



*gyr*A network for enteric bacter

But their precise branching patterns often differ

Suggesting frequent recombination within the species

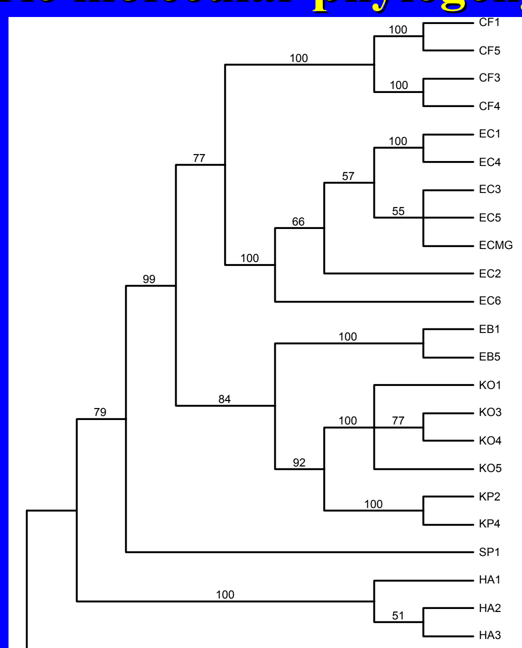


Wertz et al, submitted

Composite enteric molecular phylogeny

<u>Core genes</u>

gapA
groEL
gyrA
ompA
Pgi
16srRNA



CF
EC
EB
KO
KP
SM
HA
VC

Wertz et al, submitted

Bacterial Species Concept

- Bacteria cluster in phenotype space
- There is a corresponding genotypic clustering
  - At least for this sample of enteric bacteria
- These patterns argue for a biological species concept for bacteria and the existence of coevolved genomes that survive through evolutionary time
  - requires population as well as genomic divergence data
- The question is not “does lateral transfer occur?” but rather “does its occurrence obliterate coevolved genomes?”

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  - Robert Dorit (Yale University)
  - Junhyong Kim (Yale University)
  - Brendan Bohannon & Ben Kerr (Stanford Univ.)

Composite enteric molecular phylogeny

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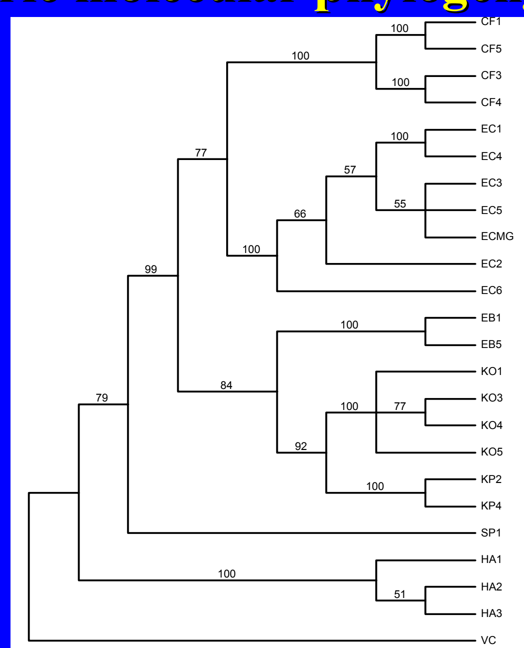

CF
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Wertz et al, submitted